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Establishment of Reference Ranges for Follitropin and Lutropin in Neonates, Infants, Children and Adolescents

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Summary: Follitropin was determined in the sera of 645 probands, and lutropin in the sera of 649 probands between the ages of 5 days and 18 years (neonates, infants, children and adolescents), using microparticle-enzyme-immunoassays (MEIA) on the IMx from Abbott Laboratories. The proband collective was divided into 9 age groups and each group into males and females. In accordance with the recommendations of the International Federation of Clinical Chemistry, the 95% scatter range was taken as the reference range. In some age groups, the ranges for individual hormones showed a significant sex difference. Age groups without significant sex differences were combined and evaluated statistically as a single group. Only a few reference groups showed a normal *Gauss*ian distribution. In addition to the 50th percentile, the 2.5th and 97.5th, or the zero and 95th percentiles were determined for all reference groups. Minimal and maximal values were also determined. The U-test of *Mann & Whitney* was used to test for significant differences between the individual reference groups. Groups showing no significant differences were combined, and reference ranges were finally calculated for follitropin and lutropin in the serum of healthy neonates, infants, children and adolescents.

Introduction

Suspected precocious or delayed maturation of the hypothalamo-hypophyseal-gonadal axis in children is an indication for the determination of serum gonadotropins.

In cases with low serum concentrations of sex steroids, the determination of gonadotropins provides evidence for the origin of a possible hypogonadism. Values above the normal range for the age of the subject point to a gonadal origin, whereas low values are indicative of a central cause. When the serum concentrations of lutropin and follitropin lie in the lower part of the reference range, or display pathologically decreased values, further differentiation can be achieved by determination of gonadotropin releasing hormones.

Even in childhood, the serum concentrations of gonadotropins display typical sex-specific changes, with two age-related maxima. During the neonatal period and in the first 6 months of life, the hypothalamo-hypophyseal-gonadal axis becomes temporarily active, with relatively high serum gonadotropin concentrations (1, 2). After the first year of life, the hypothalamo-hypophyseal-gonadal axis is suppressed. Puberty marks the onset of an episodic release of gonadotropins, which finally attains the adult male or female pattern (3, 4).

The aim of the investigation was:

1) to determine the reference ranges for follitropin and lutropin in healthy neonates, infants, children and adolescents;
2) to test for significant sex differences in the studied quantities within the age groups; and
3) to test for significant differences in the studied quantities between the age groups.

Materials and Methods

Follitropin was determined in the sera of 645 subjects, and lutropin in the sera of 649 subjects between the ages of 5 days and 18 years (neonates, infants, children and adolescents). In the course of routine screening for hypothyreosis venous blood was taken from 5-day-old neonates. For all other probands blood samples were taken after written consent was obtained from their parents, who were informed as to the purpose of the tests. The Ethics Commission of the Medical School of Erfurt gave its agreement for this purpose. The age composition of the proband collective is summarized in table 1. Individuals were included or excluded according to the exclusion criteria of *Witt & Trendelenburg* (5), which permit the assembly of a reliable reference sample at a justifiable expense. Only neonates with a birthweight between 2500 and 4000 g and a full term gestation time between 37 and 40 weeks were admitted to the 5-day-old age group. Neonates with hyperbilirubinaemia were excluded, as well as those born to mothers with acute or chronic illnesses. In sexually mature girls, blood was taken during the first 10 days of the follicular phase of a monthly cycle.

Tab. 1. Age composition of the proband collective used for the determination of reference ranges of follitropin and lutropin in neonates, infants, children and adolescents

Group		Age	Follitropin (n)	Lutropin (n)
1	♂	5th day	51	54
1	♀	5th day	53	52
1		5th day	104	106
2	♂	2–12 months	14	14
2	♀	2–12 months	11	11
2		2–12 months	25	25
3	♂	2–3 years	16	17
3	♀	2–3 years	16	16
3		2–3 years	32	33
4	♂	4–6 years	41	42
4	♀	4–6 years	23	24
4		4–6 years	63	66
5	♂	7–9 years	45	45
5	♀	7–9 years	41	41
5		7–9 years	86	86
6	♂	10–11 years	43	43
6	♀	10–11 years	53	54
6		10–11 years	96	97
7	♂	12–13 years	44	44
7	♀	12–13 years	44	41
7		12–13 years	88	85
8	♂	14–15 years	38	39
8	♀	14–15 years	37	37
8		14–15 years	75	76
9	♂	16–18 years	38	39
9	♀	16–18 years	37	37
9		16–18 years	75	75

Test material

About 2 ml of blood were taken with the informed consent of the parents, between 8.00 and 10.00 am, from an arm or skull vein, using safety monovettes from Sarstedt, Nümbrecht. Blood samples were centrifuged immediately for 5 min at 3000 min^{-1}. The serum was removed with a pipette, then frozen at −22 °C until analysed.

Methods

Follitropin and lutropin were determined by microparticle enzyme immunoassays, with the IMx from Abbott Laboratories. Cross reactivities of the tests are shown in table 2.

Tab.2. Cross reactivities reported by the manufacturer for the tests used on the IMx (tested concentrations in brackets)

Test	Cross reactivity
IMx FSH	with lutropin (1000 U/l): none with thyrotropin (2000 mU/l): none with human chorionic gonadotropin (500 000 U/l): none
IMx LH	with follitropin (2000 U/l): none with thyrotropin (2000 mU/l): none with human chorionic gonadotropin (1 000 000 U/l): 0.016%

Method sensitivities, determined on the IMx used for the present study, were 0.17 U/l for follitropin, and 0.07 U/l for lutropin.

If the serum concentrations of follitropin or lutropin lay below these respective limits, they were treated as equivalent to the value for test sensitivity (4, 6). Such test results were recorded and identified by a preceding "<".

The follitropin assay was calibrated with WHO-standard 78/549, the lutropin assay with WHO-standard 68/40.

Quality control

For the control of precision from day to day, standards (from Abbott) of low, intermediate and high concentration were included in each series (7). As a measure of the relative methodical error, the arithmetic mean ($\bar{x}$), standard deviation (s) and the variation coefficient (CV) were calculated from the individual results of these control series. Precision in series was monitored once, using the "B" and "E" calibrators (low and high concentrations) from Abbott. Again, the arithmetic mean ($\bar{x}$), standard deviation (s) and the variation coefficient (CV) were calculated from the individual results.

Statistical evaluation of the results

The results were first presented as separate histograms for each age group and for each sex. The type of distribution was determined with the *Kolmogorov-Smirnov* test. If the resulting probability error was below the stated value of $\alpha = 0.05$, the distribution was assumed to be normal. If the distribution was not normal, the 2.5th, 50th and 97.5th percentiles were determined for that reference group (8). If the 2.5th percentile lay below the sensitivity of the method, the zero, 50th and 95th percentiles were determined (9).

In each age group, the values of follitropin and lutropin were tested for significant differences between the sexes, using the U-test of *Mann & Whitney*. In the absence of a significant sex-related difference, males and females were subsequently treated as a single group. The significance of differences between age groups was also tested with the U-test of *Mann & Whitney*.

The degree of any linear relationship between age and the measured quantities was determined by calculation of the correlation coefficient, r.

Results

Follitropin

Follitropin was determined in the serum of 645 healthy probands (330 males, 315 females). Figure 1 gives an overview of the results for all groups before significance testing. Significant differences between the sexes were found in age groups 2, 3, 4, 5, 6, 7 and 8. All reference groups were tested for significant differences, using the U-test of *Mann & Whitney*. The new group combinations, formed after significance testing, are shown in table 3. The median value and reference range for serum follitropin were recalculated for each new group combination.

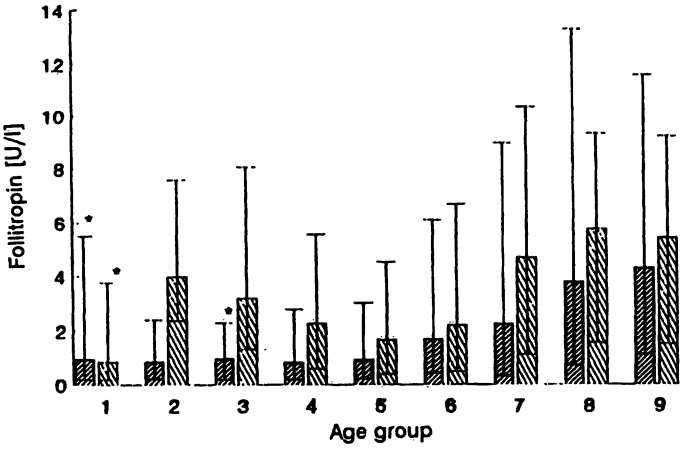

Fig. 1. 50th percentiles and 95% intervals for the concentrations of follitropin in serum (U/l) in age groups 1–9 (see table 1).
▨ = males; ▩ = females.
* Results for the interval between the zero and 95th percentile.

Five-day-old neonates did not differ significantly from males in the age range 2 months to 9 years. Since the results for neonates showed a wide scatter, and their 2.5th percentile was lower than the sensitivity of the analytical method, they were, however, treated as a separate group. Similarly, the group of 14 to 15 year-old males showed widely scattered results and was therefore treated separately, although it did not differ significantly from the 4 to 6 year-old females, the 10 to 11 year-old females, or the 12 to 13 year-old males.

Lutropin

Lutropin was determined in the serum of 649 healthy probands (336 males, 313 females). Significant differences between the sexes were found in age groups 1, 2 and 8. The U-test of *Mann & Whitney* was used to test for significant differences between the reference groups, and where possible groups showing no significant differences were combined. For each group combination made in this way, the median and reference range for serum lutropin were recalculated. An overview of the results is given in table 4 and figure 2.

Correlation analysis

Correlations with proband age were sought for the serum concentrations of follitropin and lutropin. There was a significant correlation ($p < 0.001$) between the age of the investigated probands (in months) and the concentration of each hormone, i.e. the concentrations of follicle stimulating hormone and luteinizing hormone increased with age ($r = 0.5152$ and 0.4141, respectively).

Tab. 3. 50th Percentile, 95% interval, minimal value and maximal value for the serum concentration of follitropin in neonates, infants, children and adolescents (values in U/l)

Age	Sex	n	Median (50th percentile)	Reference (95% scatter) range (2.5–97.5th percentiles)	Minimum	Maximum
5th day	♂/♀	104	0.85	<0.17– 4.59**	<0.17	12.8
2 months–9 years	♂	116	0.87	0.19– 2.7***	<0.17	3.22
2 months–3 years 12–15 years 16–18 years	♀ ♀ ♂/♀	183	4.72	1.38– 9.20	1.08	11.5
4– 6 years 10–11 years 12–13 years	♀ ♀ ♂	120	2.20	0.40– 6.61	0.30	9.39
7– 9 years 10–11 years	♀ ♂	84	1.65	0.42– 4.99	0.39	6.20
14–15 years	♂	38	3.77	0.71–13.2	0.71	13.2

** Based on the zero to 95th percentile
*** One result below the sensitivity of the test

Tab. 4. 50th Percentile, 95% interval, minimal value and maximal value for the serum concentration of lutropin in neonates, infants, children and adolescents (values in U/l)

Age	Sex	n	Median (50th percentile)	Reference (95% scatter) range (2.5–97.5th percentiles)	Minimum	Maximum
5th day 2–12 months 12–13 years	♂ ♂ ♂	112	0.65	<0.07– 3.15**	<0.07	6.94
5th day 2–12 months	♀ ♀	63	0.11	<0.07– 0.46**	<0.07	1.84
2–11 years	♂/♀	282	<0.07	<0.07– 0.39**	<0.07	2.59
12–13 years 14–15 years	♀ ♂	80	1.32	<0.07– 5.38**	<0.07	9.65
14–18 years	♀	74	3.43	0.44–12.9	0.30	17.1
16–18 years	♂	38	2.66	<0.07– 6.33**	<0.07	6.44

** Based on the zero to 95th percentile

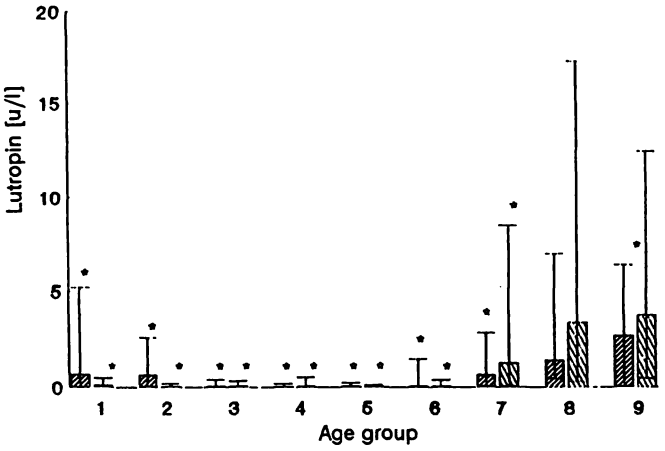

Fig. 2. 50th percentiles and 95% intervals for the concentrations of lutropin (U/l) in age groups 1–9 (see table 1). ▨ = males; ▩ = females.
* Results for the interval between the zero and 95th percentile.

Quality control

Results of the quality control are shown in tables 5 and 6. The variation coefficients within series and between series were all less than 10%.

Tab. 5. Results for the control of precision from day to day

	Control serum	n	$\bar{x}$	s	CV (%)
Follitropin (U/l)	Abbott L	31	5.09	0.35	6.88
	Abbott M	42	24.2	1.69	6.99
	Abbott H	20	79.1	5.1	6.45
Lutropin (U/l)	Abbott L	16	4.80	0.45	9.37
	Abbott M	32	42.6	2.76	6.84
	Abbott H	13	86.3	6.06	7.02

Tab. 6. Results for the control of precision in series

	Control serum	n	$\bar{x}$	s	CV (%)
Follitropin (U/l)	"B"-calibrator	23	1.04	0.05	4.49
	"E"-calibrator	20	93.8	2.97	3.17
Lutropin (U/l)	"B"-calibrator	23	2.28	0.10	4.32
	"E"-calibrator	23	101	3.42	3.39

Discussion

In addition to the anamnesis and the interpretation of clinical symptoms, the determination of hormone concentrations in serum is extremely important in the diagnosis of pathological endocrine conditions. To enable the correct evaluation of the serum concentration of a hormone, reference ranges must be established.

Modern immunological assays include the classical radioimmunoassay and several sensitive immunometric methods, e. g. MEIA, IFMA, IRMA, FPIA and time-delayed FPIA, the results of which sometimes show considerable disagreement (3, 4, 10, 11).

In the present study, microparticle enzyme immunoassays (MEIA) were used. These methods have the advantage that they yield results quickly, require only a small sample volume, and do not employ radioactive isotopes. Reference ranges for children, determined with these methods, have not been reported in the literature.

In each group, the type of distribution was determined by the *Kolmogorov-Smirnov* test. Since the reference

values in most age groups were not normally distributed, the reference range was determined by calculating the median value and the 2.5th and 97.5th percentiles, or the zero and 95th percentiles. In accordance with the recommendations of the International Federation of Clinical Chemistry, the 95% scatter range was taken as the reference range (8).

The U-test of *Mann & Whitney* was used to test for significant differences between the serum hormone concentrations of different age groups. Age groups showing no significant differences with respect to a particular hormone were usually combined and the median value and percentiles recalculated.

To facilitate comparison of the present results with those from the literature (12–14), the latter are presented in tables 7 and 8.

The reported reference ranges for the serum concentrations of follitropin and lutropin differ from those shown in tables 7 and 8 for the following reasons:

1) different methods were used;
2) the other authors used different age classifications, based partly on the puberty stages of *Tanner*;
3) the numbers of probands in the age groups of other authors were very small;
4) with the exception of *Struckmeyer & Haid* (13), the quoted authors gave no exact data on the type of distribution of the reference values.

All the quoted authors reported an increase in serum follitropin with age in both males and females, with rather higher values for females than for males of the

Tab. 7. Reference ranges reported in the literature for follitropin in serum (values in U/l)

Author	Methods	Sex	Age groups		No. of probands	Type of distribution and scatter range	Reference range
Roger in: *Ranke* 1992 (14)	No data	♂	Tanner 1	1–3 years	No clear data	No data on the distribution	1.0 (0.4–1.5)
			Tanner 1	4–9 years			0.8 (<0.3–2.4)
			Tanner 1	10–13 years			1.1 (<0.3–3.0)
			Tanner 2	11–15 years			1.5 (<0.3–3.5)
			Tanner 3/4	13–18 years			2.0 (0.6–4.8)
			Tanner 5	adult		Median value given	2.3 (0.8–4.4)
		♀	Tanner 1	1–3 years			1.5 (0.5–4.0)
			Tanner 1	4–9 years		No clear data on	1.2 (0.3–2.5)
			Tanner 1	10–15 years		the scatter range	1.7 (0.6–3.4)
			Tanner 2	11–15 years			2.0 (0.8–4.0)
			Tanner 3/4	13–18 years			3.1 (1.3–5.5)
			Menstruation	12–17 years			3.3 (1.8–5.9)
			Adult follicular phase				3.8 (2.0–6.0)
			Adult luteal phase				2.0 (0.9–4.0)
Stolecke 1992 (12)	RIA	♂	1–<3 years		No data	No data	0.4–2.7
			3–<6 years				0.6–2.3
			6–<10 years				0.8–1.9
			10–15 years				1.2–2.9
		♀	1–<3 years				0.9–3.7
			3–<6 years				1.6–3.2
			6–<10 years				1.0–3.9
			10–<12 years				1.4–4.7
			12–15 years				1.7–6.6
Struckmeyer & Haid 1986 (13)	RIA	♂	Neonates		No clear data	No normal distribution	2.75 (1.85– 4.68)
			<1 year				2.85 (1.24– 4.61)
			1–6 years				2.50 (1.30– 3.20)
			6–12 years			Median value given with 90% scatter range (5%–95% quantile)	2.8 (0.8 – 3.66)
			12–16 years				3.35 (2.09– 9.06)
		♀	Neonates				2.75 (1.58– 4.68)
			<1 year				3.70 (1.72– 6.05)
			1–6 years				3.50 (2.18– 5.15)
			6–12 years				2.60 (1.50– 5.55)
			12–16 years				6.30 (2.07–12.49)

Tab. 8. Reference ranges reported in the literature for lutropin in serum (values in U/l)

Author	Method	Sex	Age groups		No. of probands and scatter range	Type of distribution	Reference range
Roger in: *Ranke* 1992 (14)	No data	♂	Tanner 1	1–3 years	No clear data	No data on distribution	0.9 (<0.3–1.3)
			Tanner 1	4–9 years			0.8 (0.2–1.9)
			Tanner 1	10–13 years			0.7 (0.2–2.1)
			Tanner 2	11–15 years			1.0 (0.2–1.9)
			Tanner 3/4	13–16 years			1.3 (0.2–2.2)
			Tanner 5	adult		Median value given	2.0 (0.5–5.0)
		♀	Tanner 1	1–3 years			0.7 (0.2–1.4)
			Tanner 1	4–9 years		No clear data on scatter range	0.9 (0.3–2.0)
			Tanner 1	10–13 years			0.9 (0.2–2.1)
			Tanner 2	11–15 years			1.0 (0.3–2.5)
			Tanner 3/4	13–18 years			1.8 (0.4–4.5)
			Menstruation	12–17 years			2.4 (1.2–4.5)
			Adult follicular phase				2.5 (1.1–4.5)
			Adult luteal phase				1.4 (0.5–4.0)
Stolecke 1992 (12)	RIA	♂	1–<3 years		No data	No data	1.1– 2.5
			3–<6 years				0.8– 2.5
			6–<10 years				1.4– 1.9
			10–15 years				1.5– 4.9
		♀	1–<3 years				0.7– 1.5
			3–<6 years				1.3– 2.6
			6–<10 years				0.9– 2.7
			10–<12 years				1.1– 4.8
			12–15 years				1.7–12.1
Struckmeyer & Haid 1986 (13)	RIA	♂	Neonates		No clear data	No normal distribution	3.65 (0.00– 7.49)
			<1 year				1.25 (0.00– 4.78)
			1–6 years				1.25 (0.00– 3.77)
			6–12 years			Median value given with 90% scatter range (5%–95% quantile)	1.75 (0.00– 3.46)
			12–16 years				2.50 (0.00– 8.18)
		♀	Neonates				3.65 (0.00– 7.49)
			<1 year				1.85 (0.00– 4.93)
			1–6 years				0.60 (0.00– 2.35)
			6–12 years				0.00 (0.00– 2.11)
			12–16 years				7.25 (1.12–11.09)

Tab. 9. Reference ranges for follitropin and lutropin in neonates, infants, children and adolescents (values in U/l)

Follitropin:		*Lutropin:*	
Males		Males	
5th day	<0.17– 4.59	5th day	<0.07– 3.15
2 months–9 years	0.19– 2.70	2–12 months	<0.07– 3.15
10–11 years	0.42– 4.99	2–11 years	<0.07– 0.39
12–13 years	0.40– 6.61	12–13 years	<0.07– 3.15
14–15 years	0.71–13.2	14–15 years	<0.07– 5.38
16–18 years	1.38– 9.20	16–18 years	<0.07– 6.33
Females		Females	
5th day	<0.17– 4.59	5th day	<0.07– 0.46
2 months–3 years	1.38– 9.20	2–12 days	<0.07– 0.46
4– 6 years	0.40– 6.61	2–11 years	<0.07– 0.39
7– 9 years	0.42– 4.99	12–13 years	<0.07– 5.38
10–11 years	0.40– 6.61	14–18 years	0.44–12.9
12–18 years	1.38– 9.20		

same age. They all also detected two age-related maxima in the median or average follitropin values in females. The results of the last two authors (13, 14) suggest that these two follitropin maxima may also occur in males.

Serum lutropin concentrations increased with age in both males and females. All authors found higher median or average serum lutropin values in infant males. In the present study, this difference between males and females was significant in 5-day-old neonates and in infants between the ages of 2 and 12 months.

Table 9 shows the reference ranges for each hormone, as determined in the present study.

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